



Figure 1A

	SEO	SEQ I D NO:	1		<u> </u>	•		
	9	mouse_E3al1	MASEMEPEVQ	AI D- RSLLEC	SAEEI AGRWL	QAT DL NREVY	QHL AHCVPKI	49
	4	human_E3al	MASELEPEVQ	AI D- RSLLEC	SAEEI AGKWL	QAT DL TREVY	QHL AHY VPKI	49
	15	mouse_E3al	MADEEMDGAE	RIMDVSPEPPL	AP QR P AS WWD	QQV DF YT AF L	HHL AQL VPEI	50
	7	human_E3al	MADEEAGGTE	MADEE AGGTE RIVEL SAEL PQ	T P QR L AS WWD	QQVDFYTAFL	HHL AQL VPEI	50
		Consensus	MA. E	MA. E D L	A W.	Q. D.	. HLA VP. I	20
	u			L INCALL ACIT		7 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4		Ċ
	5	וומחס ב בים מוו	7		COLINE WILL			ט ע
	4	human_E3all	YCRGPNPFPQ	KEDML AQHVL	L GP ME WY L CG	EDPAFGFPKL	EQANKP SHL C.	66
	15	mouse_E3al	Y F AE MDP DL E	KOEESVOMSI	LTPLEWLFG	EDPDI CLEKL	KHSG- AFOLC	66
	7	human_E3al	YF AE MOP DL E	KQEESVQMSI	FTPLEWIFG EDPDICLEKL	EDPDI CLEKL	KHSG- AFQLC	66
		Consensus	γ	ж. 	L. P. EWYL. G. EDP KL	EDP KL	LC	100
							· ·	
•	9	mouse_E3aII	GRVFKVGEPT	YSCRDCAVDP	TCVLCMECFL GSI HRDHRYR MITSGGGGFC	GSI HRDHRYR	MITSGGGGFC	149
	4	human_E3aII	GRVFKVGEPT	YSCRDCAVDP	TCVLCMECFL	GS1 HRDHRYR	MITSGGGGFC	149
	15	mouse_E3al	GKVFKSGETT	YSCRDCAI DP	TCVL CMDCFQ	SSVHKNHRYK	MHTSTGGGFC.	149
	7	human_E3al	GRVFKSGETT	YSCRDCAI DP	TCVLCMDCFQ	DSVHKNHRYK	MHTSTGGGFC	149
		Cons ens us	GRVFK. GE. T	YSCRDCA. DP	TCVLCM CF.	. S. H HRY.	M TS. GGGFC	150
•	9	mouse_E3aII	DCGDTEAWKE	GPYCOKHKLS	SSEVVEEEDP	L VHL SEDVI A	LVHLSEDVI A RTYNI FAI MF	199
	4	human_E3aII	DCGDTE AWKE	GPYCQKHELN	TSEI.EEEEDP	L VHL SEDVI A	RTYNI FAI TF	199
	15	mous e_E3al	DCGDTEAWKT	GPFCVDHEPG RAGTTKESLH	RAGTTKESLH	- CPLNEEVI A	QARRI FPSVI	198
	7	human_E3al	DCGDTEAWKT	GPFCVNHEPG	GPFCVNHEPG RAGTI KENSR	- CPLNEEVI V	QARKI FPSVI	198
		Cons ens us	DCGDTEAWK.	GP. C HE	GP. C HE L. E. VI A	L.E.VIA		200



Figure 1B

9	mouse_E3aII	RYAVDI LTWE	KESELPEDLE	VAEKSDTYYC	ML F NDE VHT Y	RYAVDI LTWE KESELPEDLE VAEKSDTYYC MLFNDEVHTY EQVI YTLOKA	249
. 4	human_E3αII	RYAVEI LTWE	KESELPADLE	KESELPADLE MVEKSDTYYC MLFNDEVHTY	ML F NDE VHT Ÿ	EQVI YTLQKA	249
15	mouse_E3al	KYI VEMTI WE	EEKELPPELQ	KYI VEMTI WE EEKEL PPELQ I REKNERYYC VLF NDEHHSY DHVI YSLORA	VL F NDE HHS Y	DHVI YS LORA	248
7	human_E3al	KYVVE MITI WE	EEKELPPELQ	KYVVEMTI WE EEKELPPELQ I REKNERYYC VLFNDEHHSY DHVI YSLQRA	VL F NDE HHS Y	DHVI YSLORA	248
	Consensus	. Y. VE WE	. E. ELP L.	. Y. VE WE . E. ELP L EK YYC . LFNDE. H. Y VI Y. LQ. A	. LFNDE. H. Y	VI Y. LO. A	250
9	mouse_E3aII	VNCT QKE AI G	FATTVDRDGR	VNCT QKE AI G FATT V DR DGR RPVRYGDFQY CDQAKT VI VR NTSRQTK-PL	CDOAKTVI VR	NTSRQTK- PL	298
4	human_E3αII	VNCT QKE A! G	FATTVDRDGR	VNCTOKEAI G FATTVDRDGR RSVRYGDFOY CEQAKSVI VR NTSRQTK-PL	CEQAKSVI VR	NTSROTK- PL	298
15	15. · mouse_E3αl	L DCE L AE AQL	HTTAI DKEGR	LDCELAEAQL HTTAI DKEGR RAVKAGVYAT CQEAKEDI KS HSENVSQHPL	CQE AKE DI KS	HS E NV S QHP L	298
7	human_E3al	LDCELAEAQL	HTTAI DKEGR	LDCELAEAQL HTTAI DKEGR RAVKAGAYAA CQEAKEDI KS HSENVSQHPL	CQEAKEDI KS	HSENVSQHPL	298
	Consensus	C EA	T D GR	T D GR R. V G C AK I PL	C AK I	PL	300
9	mous e_E3aII	KVQVMHSSVA	AHONF GL KAL	SW.GSVI GYS	DGL RRI LCQV	KVQVMHSSVA AHQNFGLKAL SWLGSVI GYS DGLRRI LCQV GLQEGPDGEN	348
4	human_E3aII	KVQVMHSSI V	KVQVMHSSI V AHQNFGLKLL	S WL GS I I GY'S	DGLRRI LCQV	SW.GSIIGYS DGLRRILCQV GLOEGPDGEN	348
. 15	mous e_E3al	HVEVLHSVVM	AHQKF AL RLG	HVEVLHSVVM AHQKFALRLG SWMNKI MSYS SDFRQI FCQA CLVEEPGSEN	SDFRQI FCQA	CLVEEPGSEN	348
7	human_E3¤l	HVEVLHSE! M	AHOKFALRLG	S WANKI MEYS	S DF RQI F CQA	HVEVLHSEI M AHOKFALRLG SWANKI MSYS SDFROI FCOA CLREEPDSEN	348
	Cons ens us	. V. V. HS	AHQ. F. L. L.	SW I YS	R. I. CO.	. V. V. HS AHQ. F. L. L. SW I YS R. I . CQ L. E. PD. EN	350



Figure 1C

398 398 398 396	448 448 448 450
ALRFAKNYRQ AVRFAKNYQQ AMEFVKYYKQ AMEFVKYYKQ	TVI I KAF MDH SI I I KTF MDH SVI TETLLEV SVI TETLLEV SVI TETLLEV
L MOLKYKKLF L MOLKYKKLF F ME MEYKKLF F ME MEYKKLF . M YKKLF	RMLLTEENLM RMLITEENLM RHLIEEQNVI RHLIEEQNVI
VYHQLFMSSL VYHQLFMSSL I LHELI FSSF I LHELI FSSF H. L SS.	VQFFTAPTLA VQFFTAPTLA VQMLTVPTLA VQMFTVPTLA
SSLVDRLMLN DSKLWKGARS VYHQLFMSSL LMDLKYKKLF ALRFAKNYRQ SSLVDRLMLS DSKLWKGARS VYHQLFMSSL LMDLKYKKLF AVRFAKNYQQ PCLISRLMLW DAKLYKGARK I LHELIFSSF FMENEYKKLF AMEFVKYYKQ PCLISRLMLW DAKLYKGARK I LHELIFSSF FMENEYKKLF AMEFVKYYKQ LRLML. D.KL.KGARH.LSSMYKKLF AF.K.Y.Q	I LORDF MEDDH ERAVSVTALS VOFFTAPTLA RMLLTEENLM TVI I KAFMDH 448 I LORDF MEDDH ERAVSVTALS VOFFTAPTLA RMLITEENLM SI I I KTFMDH 448 LOKEYI SDDH ERSI SI TALS VOMLTVPTLA RHLI EEQNVI SVI TETLLEV 448 I LOKEYI SDDH DRSI SI TALS VOMFTVPTLA RHLI EEQNVI SVI TETLLEV 448 LO DDH ER. S. TALS VO. FT. PTLA R. LI. E. N. SVI T 450
mouse_E3all S human_E3all S mouse_E3al P human_E3al P Consensus	mouse_E3aII L human_E3aII L mouse_E3aI L human_E3aI L Consensus L
6 1 5 2	6 1 2 2 2



Figure 1D

	498	498	496	496	200	548	548	546	546	550	.598	598	595	. 595	009
	LDLKYVLI SK PTEWSDELRO	LDLKYVLI SK PTENSDELRO		_			TLOWKLTHVI	AI QMQLKNI L 5	AI QMOLKNI L	OM L			VOLCGHSLET	VQS CGHS L ET	. F CGHS. ET
	L DL KYVLI SK	LDLKYVLI SK	CDLKYI LI SK PVI WTERLRA	CDLKYI LI SK	. DLKY. LI SK PT. W LR.	EMEPEWEAAF TLOMKLTHVI	EMEPEWEAAF	E V DP. DWE A A I	EVDPDWEAAI	E. P. WE AA.	S AN QDWCALD EKVLI EAYKK CLAVLTOCHG GFT DGE QPIT LSI CGHS VET	GYTDGEOPIT	NF MS STKTV-	SEI SSSKTV-	.
	FKFRRVQSLI	FKFRRVQSLI	DKL GRVY AVI	DKL GRVY AVI	. K RV 1	PI TRQVGQHI	PI TROVGOHI	EI RROVGOHI	EI RRQVGQHI	. I . RQVGQHI	CLAVLTÖCHG	S MWQDWCAS D EKVLI EAYKK CLAVL MQCHG	L MF QE WCACD EDLLLVAYKE CHKAVMRCST NF MS STKTV-	CHKAVMRCST SFISSSKTV-	. M Q. WCA. D E L AYK. C M C
	OFERYTAL OA FKFRRVOSLI	QFERYTALOA FKFRRVOSLI	KEN- FQGYSQ DKLGRVYAVI	KFN- FQGYSQ DKL GRVYAVI	. F	ELLKCMQGMD PI TRQVGQHI	ELLKCMQGMD PI TRQVGQHI	KI LTCMQGME	KI LTCMQGME	L. CMDGM	EKVLI EAYKK	EKVLI EAYKK	EDLLLVAYKE	L MF QEWCACD EELLLVAYKE	E L AYK.
	LKHRDAQGRF	LRHRDAQGRF	LPEYLDRINN-	L PEYL DRNN-	Γ	KFLQGFDAFL	KFLEGFDAFL	QFLEGFRSFL	OF LEGFRSFL	FLEGF. FL L. CMDGM	SMYQDWCALD	S MMQDWCA:S D	L MF QE WCACD	L MF QE WCACD	. M Q. WCA. D
מבל - ס אס:	mouse_E3aII	human_E3aII	mouse_E3al	human_E3od	Consensus	mous e_E3aII	human_E3aII	mouse_E3αl	human_€3αl	Cons ens us	mous e_E3aII	human_E3αII	mous e_E3al	human_E3al	Consensus
ט ה ג	9	4	15	7		ý	4	15	7		9	4	15	7	



Figure 1E

IRYCVSQEKV SIHLPISRIL AGLHVLLSKS EVAYKFPELL PLSELSPPML 648 IYCVSQEKV SIHLPVSRLL AGLHVLLSKS EVAYKFPELL PLSELSPPML 648 KSYKVSEDLV SIHLPLSRTL AGLHVRLSRL GAISRLHEFV PFDSFQVEVL 648 KSYRVSEDLV SIHLPLSRTL AGLHVRLSRL GAVSRLHEFV SFEDFQVEVL 648Y.VSV SIHLP.SR.L AGLHV.LSE PL 650	IEHPLRCLVL CAQVHAGMMR RNGFSLVNQI YYYHNVKCRR EMFDKDIVML 693 IEHPLRCLVL CAQVHAGMMR RNGFSLVNQI YYYHNVKCRR EMFDKDVVML 693 VEYPLRCLVL VAQVVAEMMR RNGLSLISQV FYYQDVKCRE EMYDKDIIML 693 VEYPLRCLVL VAQVVAEMMR RNGLSLISQV FYYQDVKCRE EMYDKDIIML 693 VEYPLRCLVL VAQVVAEMMR RNGLSLISQV FYYQDVKCRE EMYDKDIIML 693	OTGVSMMDPN HFLM MLSRF ELYQLFSTPD YGKRFSSEVT HKDVVQQNNT 748 QTGVSMMDPN HFLM MLSRF ELYQIFSTPD YGKRFSSEIT HKDVVQQNNT 748 QIGASI MDPN KFLLLVLQRY ELTDA FNKTI STK DQDLI KQYNT 738 QIGASLMDPN KFLLLVLQRY ELAEA FNKTI STK DQDLI KQYNT 738
EVAYKFPELL EVAYKFPELL GAI SRLHEFV GAVSRLHEFV	YYYHNVKCRF YYYHNVKCRF FYYQDVKCRE FYYQDVKCRE YY. VKCR.	YGKRFSSEVT YGKRFSSEIT FNKTISTK
IRYCVSQEKV SIHLPISRLL AGLHVLLSKS EVAYKFPELL PLSELSPPNIYCVSQEKV SIHLPVSRLL AGLHVLLSKS EVAYKFPELL PLSELSPPMLKSYKVSEDLV SIHLPLSRTL AGLHVRLSRL GAISRLHEFV PFDSFQVEVKSYRVSEDLV SIHLPLSRTL AGLHVRLSRL GAVSRLHEFV SFEDFQVEV	CAQVHAGMMR RNGFSLVNQI CAQVHAGMMR RNGFSLVNQI VAQVVAEMMR RNGLSLI SQV VAQVVAEMMR RNGLSLI SQV AQV. A. MMR RNG. SL Q.	OTGVSMMDPN HFLM MLSRF ELYQLFSTPD YGKRFSSEVT OTGVSMMDPN HFLM MLSRF ELYQIFSTPD YGKRFSSEIT OIGASI MDPN KFLLLVLQRY ELTDA FNKTI STK OIGASLMDPN KFLLLVLQRY ELAEA FNKTI STK OIGASLMDPN KFLLLVLQRY ELAEA FNKTI STK
SI HLPI SRLL SI HLPVSRLL SI HLPLSRTL SI HLPLSRTL SI HLPLSRTL	CAQVHAGMMR CAQVHAGMMR VAQVVAEMMR VAQVVAEMMR · AQV. A. MMR	HFLM MLSRF HFLM MLSRF KFLLLVLQRY KFLLLVLQRY
I RYCVSQEKV I YCVSQEKV S KSYKVSEDLV KSYRVSEDLV . Y VS V	I EHPLRCLVL CAQVHAGMMR RNGFSLVNQI I EHPLRCLVL CAQVHAGMMR RNGFSLVNQI VEYPLRCLVL VAQVVAEMMR RNGLSLI SQV VEYPLRCLVL VAQVVAEMMR RNGLSLI SQV . E. PLRCLVL . AQV. A. MMR RNG. SL Q.	QT GVS MMDPN QT GVS MMDPN QI GASI MDPN QI GASI MDPN
mous e_E3aII huma n_E3aII mous e_E3aI huma n_E3aI Cons e ns us	mouse_E3αII human_E3αII mouse_E3αI human_E3αI Consensus	mous e_E3aII huma n_E3aII mous e_E3aI huma n_E3aI
6 1 1 5 2 2	2 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	6 1 2 2 . 2 .



Figure 1F

798 798 788 788 800	848 848 838 838 850	898 898 888 888
KP MAHS EL VK KP MAHS EL VK EP MP HS AI AR EP MP HS AI AK . PM HSK	KEFNLYFYHF KEFNLYFYHF KDFNMYFYHY KDFNMYFYHY K. FN. YFYH.	VNI LOCDVML VNI LOSDVML VNI LSCDVM I NLLNCDI MM VN. L. CDVM
KREI I HQL SI KREI I HQL SI MREI THL LCI MREI I HL LCI . REI I H. L. I	GMYELKPECA GMYELKPECA GVYELKDESL I GVYELKDESL G. YELK. E.	PPFCPLFASL PPFCPLFASL PEFCPAFSKV PEFCPAFSKV PFFCPAFSKV
LIEEMLYLII MLVGERFNPG VGQVAATDEI KREIIHQLSI LIEEMLYLII MLVGERFSPG VGQVNATDEI KREIIHQLSI LIEEMLQVLI YIVGERYVPG VGNVTREEVI MREITHLLCI LIEEMLQVLI YIVGERYVPG VGNVTKEEVT MREIIHLLCI LIEEMLIVGERPG VG.VI .REIIH.L.I	SLPEDENKET GMESVIESVA HFKKPGLTGR GMYELKPECA KEFNLYFYHF SLPEDENKET GMESVIEAVA HFKKPGLTGR GMYELKPECA KEFNLYFYHF NLPENENNET GLENVINKVA TFKKPGVSGH GVYELKDESL KDFNMYFYHY NLPENENNET GLENVINKVA TFKKPGVSGH GVYELKDESL KDFNMYFYHY NLPENENNET GLEVINKVA TFKKPG.G.G.YELK.E.K.FN.YFYH.	EDTALPPPAL EDTALPPPVL KDEALPPPPP KDEALPPPPP
MLVGERFSPC MLVGERFSPC YIVGERYVPG YIVGERYVPC	GNESVIESVA GNESVIEAVA GLENVINKVA GLENVINKVA	SRAEQSKAEE AQRKLKRENK SRAEQSKAEE AQRKLKRQNR SKTQHSKAEH MQKKRRKQEN SKTQHSKAEH MQKKRRKQEN SSKAE. Q.KQ.
LI EEMLYLII LI EEMLYLII LI EEMLQVLI LI EEMLQVLI	SLPEDENKET SLPEDENKET NLPENENNET NLPENENNET . LPE. EN. ET	SRAE QS KAEE SRAE QS KAEE SKT QHS KAEH SKT QHS KAEH
mouse_E3aII human_E3aII mouse_E3aI human_E3aI Consensus	mous e_E3aII human_E3aII mous e_E3aI human_E3aI Cons ens us	mous e_E3aII huma n_E3aII mous e_E3aI huma n_E3aI Cons ens us
2 1 5	6 1 5 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 	6 4 4 5 5 · 2 2 · 3 · 3 · 3 · 3 · 3 · 3 · 3 · 3 ·



Figure 1G

948 948	937	950	866	866	987	984	1000	1046	046	037	1034	050
L E NAVE GHVQ L E NVT E E HVV	L QKAPEEEV- L OKAPEEEV-	L. A. EE. V.	I RWLLKMFNA	I RW LKTFNA	I TW LOWFDT	I TW LOWF DT		I ARL RREKI M 1046	I ARLRREKI M 1046	AARLHRQKI M 1037	AARLHRQKI M 1034	. ARL. R. KI M 1050
GMALQEEKHH LENAVEGHVQ GMALQEEKQH LENVTEEHVV	ALGLLEEKQQ LOKAPEEEV-	YI TI A V W.E . MLQ H L. EEKQ. L A. EE. V.	TFTFTQKISK PGDAPHNSPS I LAMLETLQN APSLEAHKDM I RWLLKMFNA	TFTFTQKISK PGEAPKNSPS I LAMLETLQN APYLEVHKDM I RW LKTFNA	AFDFYHKASR LGSSAMNAQN I QMLLERLKG I PQLEGQKDM I TWILQMFDT	TFDFYHKASR LGSSAMNI QM L LEKLKG I PQLEGQKDM I TW LQMFDT	TF. F K. S G N I LE. L P. LE KDM I . WI L. MF	DKAERKRKAE	DKAERKRKAE		EKAERKRKAE	. KAERKRKAE
S ML QRVL HL I S ML QRVL HL I	GML QMAFHIL	M O	ILAMLETLON	ILAMLETLON	IOMLLERLKG	LLEKLKG	I LE. L	I KKI RE CS SSSPVAEAEG TI MEESSRDK	TI MEESSRDK	VKRLREKSCL VVATTSGLEC I KSEE! THDK EKAERKRKAE	VKRLREKSCL I VATTSGSES I KNDEI THDK EKAERKRKAE	E EE DK . KAERKRKAE
YIMGTILQWA VEHHGSAWSE SMLQRVLHLI CIMGTILQWA VEHNGYAWSE SMLQRVLHLI	YILRTIFERA VDTESNLWTE GMLOMAFHIL YILRTVFERA IDTDSNLWTE GMLOMAFHIL	VW.E	P GDAPHNS P S	PGEAPKNSPS	L GS S AMNAQN	LGS S AMNI QM	. G R	SSSPVAEAEG	PTSPVAETEG	VVATTSGLEC	I VATTSGSES	
YI MGTI LQWA CI MGTI LQWA	YI LRTI FERA YI LRTVFERA	YI TI A	TFTFTQKI SK	TFTFTQKI SK	AF DF Y HKAS R	TFDFYHKASR	TF. F K. S.	I KKI RECS	VKKMRESS	VKRLREKSCL	VKRLREKSCL	VK RE C.
SEQ I D NO: 6 mouse_E3aII 4 human_E3aII	mouse_E3al	Consensus	mouse_E3all	human_E3aII	mouse_E3αl	human_E3al	Cons ens us	mous e_E3aII	human_E3al	mouse_E3αl	huma'n_E3αl	Cons ens us
SEQ 6 4	15))	. •	4	15	7		9	4	15	7	



Figure 1H

1094 1094 1087 1084 1100	1144 11137 1134 1150	1193 1193 1184 1181
AQMSEMQRHF I DENKELFQQ TLELDTSASA TLDSSPPV SDAALTALGP 1094 AQMSEMQRHF I DENKELFQQ TLELDASTSA VLDHSPVA SDMTLTALGP 1094 AQMSALQKNF I ETHKLMYDN TSEVTGKEDS I MEEESTSAV SEASRI ALGP 1087 AQMSALQKNF I ETHKLMYDN TSEMPGKEDS I MEEESTPAV SDYSRI ALGP 1084 AQMS. Q. F I K T. E S. P. V SD ALGP 1100	VLSKDRTKTI VLSKNRSKFI ALTQHRGKPV ALTQHRGKPI	AD-PEKYDPL FMHPDLSCGT HTGSCGHVMH AHCWQRYFDS VQAKEQRRQQ 1193 QD-PEKYDPL FMHPDLSCGT HTSSCGHI MH AHCWQRYFDS VQAKEQRRQQ 1193 DHLGETLDPL FMDPDLAHGT YTGSCGHVMH AVCWQKYFEA VQLSSQQ 1181 ELSGEALDPL FMDPDLAYGT YTGSCGHVMH AVCWQKYFEA VQLSSQQ 1181 CLSGEALDPL FMDPDLAYGT YTGSCGHVMH AVCWQKYFEA VQLSSQQ 1181 CLSGEALDPL FM PDL. GT . TGSGGHVMH A.CWQ.YF. VQ
TLDSSPPV VLDHSPVA I MEEESTSAV I MEEESTPAV	VLAAFVQRST VLAAFVQRST VLSACVQKST VLSACVQKST VLSACVQKST	AHCWQRYFDS AHCWQRYFDS AVCWQKYFEA AVCWQKYFEA
TLELDTSASA TLELDASTSA TSEVTGKEDS TSEMPGKEDS T.E	QEVTVGSRAM QEVKVESRAM QEVKLENNAM QEVK!ENNAM QEVK!ENNAM	HT GS CGHVMH HT S S CGHI MH YT GS CGHVMH YT GS CGHVMH
I DENKELFQQ I DENKELFQQ I ETHKLMYDN I ETHKLMYDN I K	AQTQVPEPRQ FVTCI LCQEE QEVTVGSRAM VLAAFVQRST VLSKDRTKTI TQTQVPEQRQ FVTCI LCQEE QEVKVESRAM VLAAFVQRST VLSKNRSKFI KRGPAVTEKE VLTCI LCQEE QEVKLENNAM VLSACVQKST ALTQHRGKPV KRGPSVTEKE VLTCI LCQEE QEVKI ENNAM VLSACVQKST ALTQHRGKPI	F MHP DL S CGT F MHP DL S CGT F MDP DL A HGT F MDP DL A Y GT F M P DL GT
AQMS E MQRHF AQMS E MQRHF AQMS AL QKNF AQMS AL QKNF AQMS Q F	AQTQVPEPRQ TQTQVPEQRQ KRGPAVTEKE KRGPSVTEKE	AD- PEKYDPL QD- PEKYDPL DHLGETLDPL ELSGEALDPL
mouse_E3αII human_E3αII mouse_E3αI human_E3αI Consensus	mous e_E3aII human_E3aII mous e_E3aI human_E3a Cons ens us	mous e_E3aII human_E3aII mous e_E3aI human_E3aI
6 1 2 2	4. 1. 2.	6 1 5 2



Figure 11

1241 1241 1233 1230 1250.	1290 1290 1283 1279 1300	1340 1340 1333 1329 1350
L-LPPRSILS RRLN-FSDQP L-LPPRNIFN NRLN-FSDQP IPLQPQKINS ENAEALAQLL IPLQPQKINS ENADALAQLL	SSEDTEAMNI IPIPEGFRPD 1290 STKNSENVDE LOLPEGFRPD 1290 LFNQGMGDST FEFHSILSFG 1283 FFNQGMGDST LEFHSILSFG 1279	LCWGTCAYT! MCWGSCAYT! MTWSTCAFT! LTWSTCAFT!W.TCA.T!
	QQI KVVQMLR RKHNAA-DTS SSEDTEAMNI I PI PEGFRPD 1290 QQI KALQFLR KEESTP-NNA STKNSENVDE LQLPEGFRPD 1290 ARI SGYNI KH AKGEAPAVPV LFNQGMGDST FEFHSI LSFG 1283 ARI SGYNI RH AKGENP-I PI FFNQGMGDST LEFHSI LSFG 1279	PNEGDPRVPI LCWGTCAYTI PNEEDPRVPI MCWGSCAYTI PDELDPRVPM MTWGTCAFTI PDERDPRVPM LTWSTCAFTI P. E. DPRVP W TCA. TI
ENGEFLCPLC ECLSNTVI PL ENGEFLCPLC ECLSNTVI PL ESGEYLCPLC KSLCNTVI PI ESGEYLCPLC KSLCNTVI PI E. GE. LCPLC L. NTVI P.	QQI KVVQMLR RKHNAA- DTS SSEDTEAMNI QQI KALQFLR KEESTP- NNA STKNSENVDE ARI SGYNI KH AKGEAPAVPV LFNQGMGDST ARI SGYNI RH AKGENP- I PI FFNQGMGDST	FYPRNPYSDS I KEMLTTFGT AAYKVGLKVH FRPKI PYSES I KEMLTTFGT ATYKVGLKVH VQSSVKYSNS I KEMVI LFAT TI YRI GLKVP VESSI KYSNS I KEMVI LFAT TI YRI GLKVP YS. S I KEM F. T Y GLKV.
	QQI KVVQMLR QQI KALQFLR ARI SGYNI KH ARI SGYNI RH	FYPRNPYSDS I KEMLTTFGT AAYKVGLKVH FRPKI PYSES I KEMLTTFGT ATYKVGLKVH VQSSVKYSNS I KEMVI LFAT TI YRI GLKVP VESSI KYSNS I KEMVI LFAT TI YRI GLKVP ······YS. S I KEM. · F. T ···Y. · GLKV.
RLRLHTSYDV RLRLHTSYDV RI HVDL - FDL RI HVDL - FDL R D.	DLAQWIRAVT NLTQW RT! S TLARW QTVL TLARW QTVL .LA. W. TV.	FYPRNPYSDS FRPKI PYSES VQSSVKYSNS VESSI KYSNS
muse_E3aII human_E3aII muse_E3aI human_E3aI Consensus	mous e_E3aII huma n_E3aII mous e_E3aI huma n_E3aI	mous e_E3all huma n_E3all mous e_E3al huma n_E3al Cons ens us
6 1 2	6 4 4 2 2 2	6 1 5 2



Figure 1J

SEQ ID NO:	1 6 0 1					1
6 mouse_E3aII	QSIERILSDE	EKPVFGPLPC	RLDDCLRSLT	RFAAAHWTVA	LLPVVQGHFC	1390
4 human_E3¤II	QSIERILSDE	DKPLFGPLPC	RLDDCLRSLT	RFAAAHWTVA	SVSVVQGHFC	1390
15 mouse_E3αI	QAIENLLGDE	GKPLFGALQN	RQHSGLKALM	QFAVAQRATC	PQVLIHKHLA	1383
2 human_E3¤I	QAIENLLGDE	GKPLFGALQN	RQHNGLKALM	QFAVAQRITC	PQVLIQKHLV	1379
Consensus	Q. IE L.DE	. KPLFG.L	RLL.	. FA . A	Q . н.	1400
6 mouse_E3aII	KLFASLVPSD	SYEDLPCILD	IDMFHLLVGL	VLAFPALQCQ	DFSGSSL	1437
4 human_E3all	KLFASLVPND.	SHEELPCILD	IDMFHLLVGL	VLAFPALQCQ	DFSGISL	1437
15 mouse_E3aI	RLLSVILPNL	QSENTPGLLS	VDLFHVLVGA	VLAFPSLYWD	DTVDLQPSPL 1433	1433
2 human_E3 \alpha I	RLLSVVLPNI	KSEDTPCLLS	IDLFHVLVGA	VLAFPSLYWD	DPVDLQPSSV 1429	1429
Consensus	. L PN.	E PC .L .	ID. FH. LVG .	VLAFP. L	D SSL	1450
mouse_E3aII	ATGDLHIF	HLVTMAHIVQ	ILLTSCTEEN	GMDQENP	TGEEELAILS	1482
4 human_E3aII	GTGDLHIF	HLVTMAHIIQ	ILLTSCTEEN	GMDQENP	PCEEESAVLA	1482
15 mouse_E3al	SSSYNHLYLF	HLITMAHMLQ	ILLTTDTDLS	PGPPLAEGEE	DSEEARCASA	1483
2 human_E3αI	SSSYNHLYLF	HLITMAHMLQ	ILLTVDTGL-	PLAQVQE	DSEEAHSASS	1475
Consensus	F	HL. TMAH Q ILLT T	ILLTT	0	EE	1500



Figure 1K

FHYLNGVPAP 1532 FHYLNGVPSP 1532 FHYLLGVAPP 1533 FHYLLGVTPP 1525 FHYLLGVTPP 1525	ESWCQNSEVK 1581 ESWCRNSEVK 1581 QRWCGDPALL 1583 QRWCADPALL 1575WC 1600	APT 1631 APT 1631 HPV 1633 HPV 1625
FHYLNGVPAP 1532 FHYLNGVPSP 1532 FHYLLGVAPP 1533 FHYLLGVTPP 1525 FHYL GVP 1550	ESWČQNSEVK 1581 ESWCRNSEVK 1581 QRWCGDPALL 1583 QRWCADPALL 1575	SGGDKSR SGGDKSR SADDERK SADDERK SD
MPFLKCSAL MPFLKCSALF TPYLRCAALL TPYLRCAALF	LPTNLIHLFQ ENSDIMNSLI ESWCQNSEVK 1581 LPNNLICLFQ ENSEIMNSLI ESWCRNSEVK 1581 LPTNLFLLFQ EYWDTIRPLL QRWCGDPALL 1583 LPTNLFLLFQ EYWDTVRPLL QRWCADPALL 1575 LPTNLFLLFQ EDLWC 1600	NQASNFSCPK SGGDKSRAPT 1631 NQASNFSCPK SGGDKSRAPT 1631 NQASHFRCPR SADDERKHPV 1633 NQASHFRCPR SADDERKHPV 1625 NQAS.F.CP. SDP. 1650
SALKEAPSGW HLWRSVRAAI MPFLKCSAL SALKEIPSGW HLWRSVRAGI MPFLKCSALF GLTGCGAPGW YLWLSLRNGI TPYLRCAALL GSIGCDIPGW YLWVSLKNGI TPYLRCAALF	LPTNLIHLFQ LPNNLICLFQ LPTNLFLLFQ LPTNLFLLFQ LPTNLLFQ	DLPEDYSSLI NLPEDYSSLI ELPEDYSCLL ELPDDYSCLL . LPEDYS . L.
SALKEAPSGW SALKEIPSGW GLTGCGAPGW GSIGCDIPGW	HFEHLCNYLS HFEHLCSYLS EFSALCSYLS EYSALCSYLS . F LCSYLS	SYPRGANKLI RYPRESNKLI RYPRKRNSLI RYPRKRNSLI RYPRN.LI
LHKTLHQYTG LYKTLHQYTG FFVEVSQHTD FFAEISQYTS	PDLQV-SGTS PDIQV-PGTS EELFANSAEG EELHTNSAEG	RYLNGERGAI RYLEGERDA! KSLKQKSAVV NCLKQKNTVV
6 mouse_E3all 4 human_E3all 15 mouse_E3al 2 human_E3al Consensus	6 mouse_E3aII 4 human_E3aII 15 mouse_E3aI 2 human_E3aI	6 mouse_E3aII 4 human_E3aII 15 mouse_E3aI 2 human_E3aI



Figure 1L

1681 1681 1683	1675	1731	1733 1725 1750		
LCLVCGSLLC SQSYCCQAEL EGEDVGACTA HTYSCGSGAG IFLRVRECQV 1681 LCLVCGSLLC SQSYCCQTEL EGEDVGACTA HTYSCGSGVG IFLRVRECQV 1681 LCLFCGAILC SQNICCQEIV NGEEVGACVF HALHCGAGVC IFLKIRECRV 1683	I FLKI RECRV I FL REC. V	RKI QKL WQQH KKI QKL WHQH	RKL HL VWQQH RKL HL VWQQH RK WQQH	·	· .
HTYSCGSGAG HTYSCGSGVG HALHCGAGVC	HAL HCGAGVC H CG. GV.	NPL HL CQERF NPL HL CKERF	NPLHLSRERY NPLHLSRERY NPLHL.ER.	1755 1755	1757 1749 1774
EGE DVGACT A EGE DVGACT A NGE EVGACVF	NGEEVGACI F . GE. VGAC	GET DOGL RRG GET DOGL RRG	GETDPGLKRG GETDPGLKRG GETD. GL. RG		
SQSYCCQAEL SQSYCCQTEL SQNI CCQEIV	SONI CCOEI V SQ CCQ	FYSPPYLDDY FYSPPYLDDY	AYPAPYL DEY AYPAPYL DEY . Y PYL D. Y	EANQT LVGI D EANQT LVGI D	ETNOMLFGFN ETNOMLFGFN E. NO. L. G.
LCLVCGSLLG SQSYCCQAEL EGEDVGACTA HTYSCGSGAG IFLRVRECQV 1681 LCLVCGSLLC SQSYCCQTEL EGEDVGACTA HTYSCGSGVG IFLRVRECQV 1681 LCLFCGAILC SQNICCQEIV NGEEVGACVF HALHCGAGVC IFLKIRECRV 1683	LCLFCGAILC SQNI CCQEIV NGEEVGACIF HALHCGAGVC IFLKIRECRV 1675 LCL. CG LC SQ CCQ GE. VGAC H CG. GV. IFL REC. V 1700	LFLAGKTKGC FYSPPYLDDY GETDQGLRRG NPLHLCQERF RKI QKLWQQH 1731 LFLAGKTKGC FYSPPYLDDY GETDQGLRRG NPLHLCKERF KKI QKLWHQH 1731	VLVEGKARGC AYPAPYLDEY GETDPGLKRG NPLHLSRERY RKLHLVWQQH 1733 VLVEGKARGC AYPAPYLDEY GETDPGLKRG NPLHLSRERY RKLHLVWQQH 1725 GKGC . Y PYLD. Y GETD. GL. RG NPLHL ER. RK WQQH 1750	SI TEE! GHAQ EANQTLVGI D WQHL SVTEE! GHAQ EANQTLVGI D WQHL	CI I EEI ARSQ ETNOMLFGFN WOLL CI I EEI ARSQ ETNOMLFGFN WOLL I . EEI Q E. NQ. L. G WO. L.
mous e_E3αII huma n_E3αII mous e_E3αI	human_E3al Consensus	mouse_E3aII human_E3aII	mous e_E3al huma n_E3al Cons ens us	mouse_E3aII human_E3aII	
6 15	2	0 4	15	0 4	15



FIG. 2

Tth Expression Profile of huE3 α -II in Human Tissues

Brain
Heart
Skeletal muscle
Colon
Thymus
Spleen
Kidney
Liver
Small intestine
Placenta
Lung
Leukocyte

9.5kb ---

7.5kb —

4.4kb —

2.4kb —



FIG. 3

Tth Expression Profile of huE3 α -l in Human Tissues

Heart Brain Placenta Lung Liver Skeletal Musck Kidney

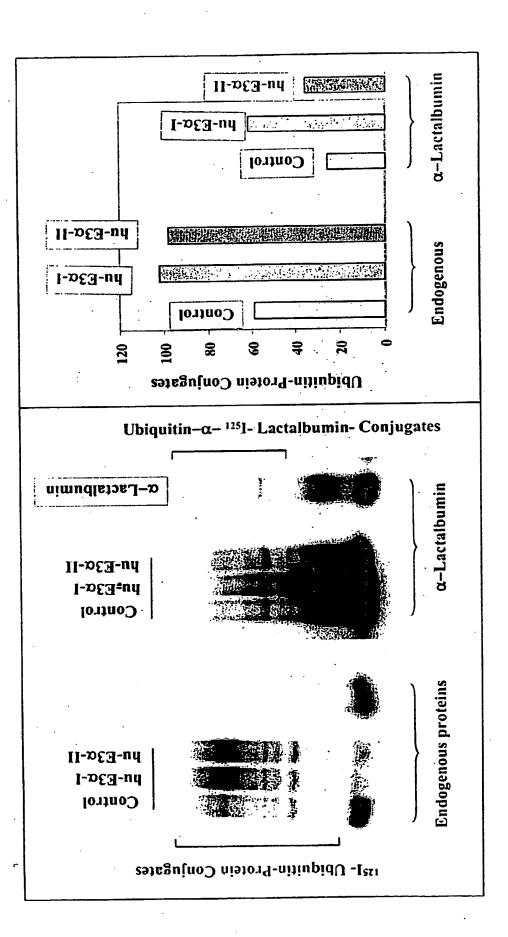
9.5kb __ 7.5kb __

4.4kb —

2.4kb ---

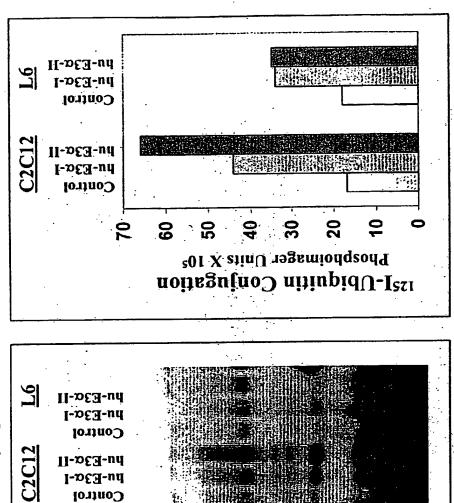


Figure 4
Ubiquitination of Endogenous Proteins



Transfection of Human E3a-I or E3a-II cDNA Stimulates Ubiquitin Conjugation in Cultured Muscle Cell Lines Figure 5

hu-E3a-l Control



Conjugates

125I-Ubiquitin-Protein



Figure 6

 $^{125}\text{I-Ubiquitin}$ Conjugation to Muscle Proteins and Its Sensitivity to E3 α Inhibitor in Skeletal Muscle Extracts

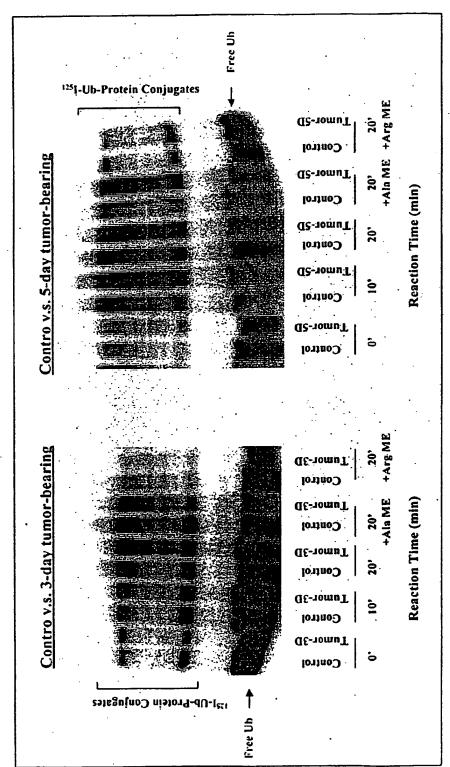




Figure 7

Rates of Ubiquitination of N-end Rule Substrate α-Lactalbumin in Skeletal Muscle Extracts

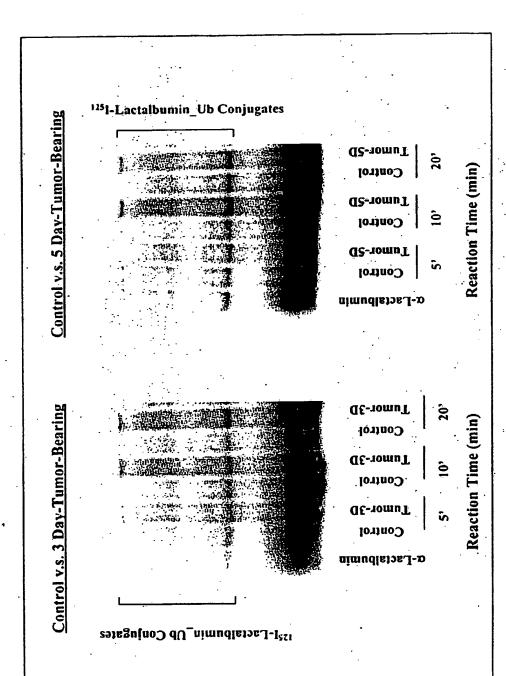




Figure 8

Northern blot analysis of E3 α -I & E3 α -II expression



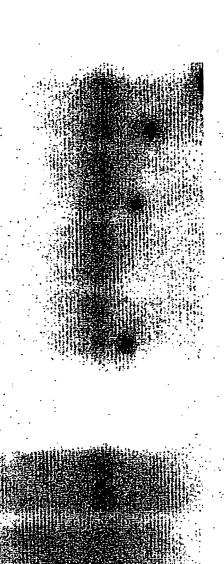


Figure 9

Northern blot analysis of E3 α -I and E3 α -II expression in gastrocnemius muscle and cardiac muscle in C26 experimental cachexia model

	tumor, d17		
•	71b ,b91 inag		
E3α-1	control, d17		
	. to nomut		
	pari fed, dl2		
	control, d12		1
	tumor, d17		
	Pari fed, dl7		
F30-11	control, d17		
	tumor, dl2		
	pari fed, d12		
	control, dl2		
٠	·	Gastroenmius	Heart



Figure 10

induce E3α-II Expression in C2C12 myostube culture Proinflammatory cytokines TNF- α and IL-6

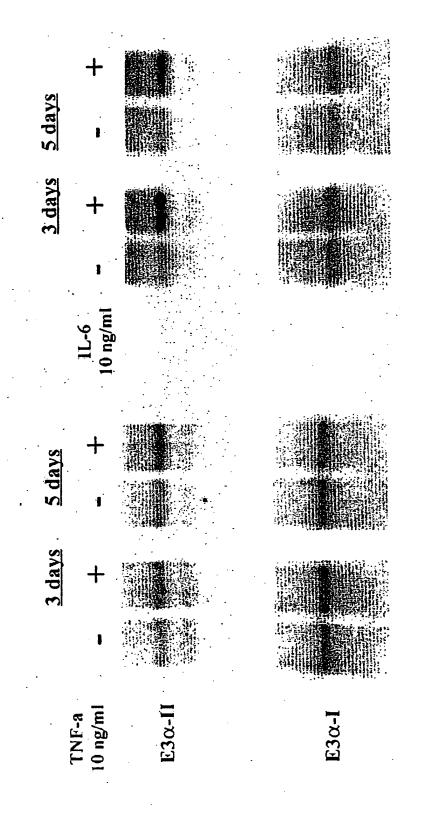
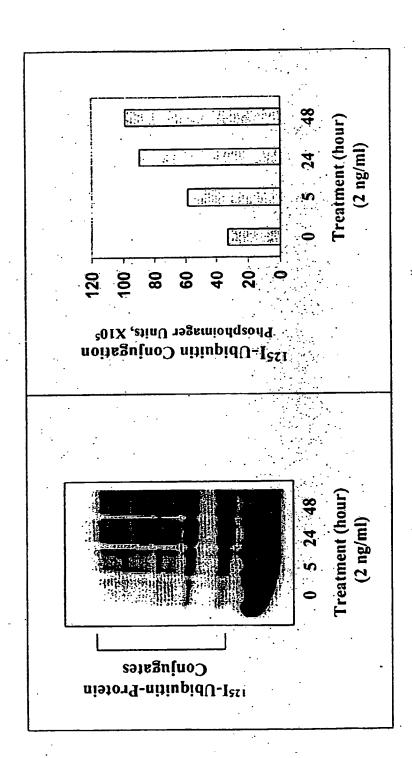




Figure 11

IL-6 Elicits Accelerated Ubiquitination in C2C12 Myotube Cultures



PE STEE

 ${\sf TNF}_{\alpha}$ Elicits Accelerated Ubiquitination in C2C12 Myotube Cultures Figure 12

